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Summary

A new study in fission yeasts promotes the notion that transient polarity patches that wander the cell surface at the onset of mating are discrete agents of gradient sensing. This concept unexpectedly bridges the modes of gradient sensing in eukaryotes and prokaryotes.

The ability of cells to sense gradients of relevant environmental factors and align the direction of migration (chemotaxis) or growth (chemotropism) along these gradients is critical for their life functions and survival. Detailed molecular mechanisms and general biophysical principles of gradient sensing are thus of much interest and have remained the focus of continuous experimental and theoretical efforts for decades. The general consensus has been that prokaryotes and eukaryotes utilize distinct biophysical strategies. Temporary sampling of chemical gradients via biased random walk has been firmly established as the principle of chemosensing for small bacterial cells [1]. In contrast, the field of eukaryotic chemotaxis, led by the studies on large motile cells, such as *Dictyostelium* amoeba and neutrophils [2], concluded that eukaryotic cells sense gradients spatially, by detecting differences in the occupancy of chemosensory receptors along their surface. However, it remained unclear how small eukaryotes, such as yeasts, manage to accurately determine the direction of signalling gradients [3] despite their size that places them closer to the prokaryotic world. Recent studies performed at a higher spatial and temporal resolution revealed a trial-and-error process that underlies gradient sensing in budding and fission yeasts and their conclusions began to question the validity of the spatial gradient sensing hypothesis in these unicellular fungi [4-7]. In this issue, Merlini et al. [8] approach fission yeast mating with an engineering perspective and conclude that the biophysical principle of gradient sensing in this organism may, after all, be not too different from the strategy employed by the prokaryotic microbes.

Under inducing conditions, fungal haploid cells secrete mating pheromones and express receptors for the pheromone of the opposite sex. During mating, cells pair up by sensing the secreted pheromones, grow towards each other by forming mating protrusions, a.k.a. shmoos, and eventually fuse to form a diploid zygote. In filamentous fungi, in addition to this sexual process, also vegetative cells fuse to form large branching colonies. This process, best understood in *Neurospora crassa* [9, 10], involves chemotropism between genetically identical cells and, therefore, is mediated by a common signalling molecule. To avoid self-activation, *N. crassa* evolved a peculiar “Ping-Pong” mechanism of pulsatile signal exchange that ensues between the paired up cellular protrusions during their homing towards each other [11, 12].

Studies in budding yeast mating have been instrumental to inform the efforts in eukaryotic gradient sensing for over three decades [13]. Early work mainly focused on three molecular modules directly responsible for pheromone sensing: pheromones and their secretory machinery, receptors and associated G-proteins, as well as the downstream Fus3 MAP kinase cascade. Research in the Herskowitz and Peter labs also identified the molecular link between the pheromone-induced G-protein signalling and the emergence and growth of the shmoo [14] via the activation of small GTPase Cdc42, a master-regulator of eukaryotic cell polarity. Thus it has been assumed that the polarity module that controls chemotropic growth is activated downstream of choosing the direction towards the mating partner. Recent progress in fluorescence microscopy and the development of fluorescent proteins and sensors for activated GTPases provided evidence that calls for the revision of this concept. Papers from the Lew and Martin groups [4, 5] revealed that prior to the shmooing proper, i.e., the phase of committed polarized growth towards the mating partner, there exists a transient exploratory phase during which a weak polarity patch wanders the cell surface as if unsure of the right direction. Interestingly, in both budding and fission yeasts, these transient patches were smaller and weaker in fluorescence intensity than the polarity clusters observed during the vegetative polarized growth. Peter and colleagues [7] concluded that this property is the cause for the dynamic, wandering nature of an exploratory patch whereas its intensification in response to stronger pheromone signal is a prerequisite for the conversion of a patch into the polarity cluster proper that drives the protrusion of the shmoo. Like in the context of budding, emergence of the exploratory patch was shown to be independent of actin cytoskeleton [4, 6]. This confirmed that, both in budding and mating, the discrete polarity domain is a self-organized structure and it arises via positive feedback

encoded by the molecular interactions within the Cdc42 polarity module [15-17]. In contrast, mobility of the exploratory polarity patch was found largely actin-dependent and negatively regulated by the strengthening of the Cdc42 positive feedback [4, 6]. Unlike in budding yeast, Martin and colleagues observed that, in fission yeast, the exploratory patch moves by large jumps, disassembling in one spot and re-assembling in the other [5].

In their new contribution, Merlini et al. [8] first address a question that naturally flows from the earlier studies: Is the exploratory polarity patch actually a locus of signal release and signal sensing? Starting with a demonstration of co-localization of the patch with some components of the secretory and sensory machineries, they proceed to analyze mating efficiency in a set of computational models varying in the scope (total cell surface vs. only the patch) of signal sensing and signal releasing. In this analysis they rely on a powerful quantitative assay that allows them to measure the kinetics of yeast mating (as the percentage of paired cells vs. time) under various perturbations and compare the results with model predictions. Their conclusion is clear – the model where the release and sensing of the pheromone are co-localized with the polarity patch outperforms other models and its simulated mating efficiency reaches that of the wild type yeast population. It is interesting that in *N. crassa* the analogs of the polarity patches detected by the activity of Cdc42 and Rac-1 coincide with the sites of signal reception as reported by the localization of signal-responding MAPK Mak-2 (Fus3 homologue) [18, 19]. It is tempting to speculate that, even in large filamentous fungi, there exist localized zones of heightened signal sensing (and, very likely, release) that coincide with and are defined by the self-organized clusters of GTPase activity. In support of this conjecture, chemical inhibition of Rac-1 activity resulted in cessation of pulsed Mak-2 recruitment [19].

Merlini et al. [8] further ask if patch mobility, which in their experimental system is essentially determined by the patch lifetime, affects the mating efficiency. Experimental increase in the patch lifetime reduced the efficiency of mating by increasing the proportion of cells that formed unreciprocated shmoos. Likewise, model analysis suggested existence of the optimal patch lifetime that maximizes the efficiency of mating. These results are in a good qualitative agreement with budding yeast studies where both overly mobile and static patches were found detrimental [4, 6, 7].

Taken together, the novel results of Martin, Vavylonis and colleagues published in this issue of Current Biology [8] strengthen the concept of transient polarity patches as discrete platforms for gradient sensing. Cumulative evidence from several fungal systems suggests that these platforms are equipped with signal sending and signal receiving capabilities and can translocate along the surface of cells either gradually, as reported in budding yeast cells, or in jumps due to the repeating cycles of assembly/disassembly, as observed in larger cells of fission yeast and filamentous fungi. Their discrete nature and characteristic size, which varies across fungi much less than the size of the cells themselves, are likely determined by the pattern-forming ability of Cdc42 and other small GTPases involved. Given that the nature of the exploratory patches is akin to those of vegetative polarity clusters, it remains to be seen if competition for the common molecular resources that ensures the uniqueness (a.k.a. singularity) of the yeast bud [17, 20] also provides for the unique exploratory patch per mating cell. As in the case of budding, fusing with multiple mating partners would be unproductive due to the uniqueness of the yeast nucleus. This is not a restriction in the multi-nucleated cells of *N. crassa*, where formation of multiple signalling patches per cell has been indeed observed. Considering an exploratory phase patch as an independent agent of a sub-micron size that performs a biased random walk, constrained only by the surface of the "host" cell, makes for a surprising analogy between the gradient sensing strategies of bacteria and fungi (see Figure 1). Indeed, in both cases the agent (bacterial cell or sensing patch) moves randomly in the complex spatial profile of the signalling molecule, biasing its walk towards the increase in signal amplitude and thus using the temporal averaging strategy, previously attributed solely to bacteria. In case of populations of mating or fusing

vegetative cells, such a walk is bound to have multiple attractors and frequent switches of partners have indeed been seen among cells of fission yeasts and *N. crassa*. Remarkably, in this novel hybrid mechanism, the eukaryotic cell still utilizes its entire cell surface. However it does so by supporting a random walk of a searching agent rather than by attempting a complex and error-prone calculation of receptor occupancy differences. The thought-provoking study by Merlini et al. [8] promotes our understanding of eukaryotic gradient sensing and vividly illustrates the power of the systems biology approach towards complex biological problems.

Figure 1. Fission yeasts play “Hot and Cold” dating game during the exploratory phase of mating. (A) Transient polarity patches that emit mating pheromone and sense pheromone of the opposite sex (shown as colored gradients) undergo a biased random walk on the cell surface to establish efficient cell pairing. (B) Following a similar strategy, bacterial cells sense chemical gradients via a random, run-and-tumble motion that, on average, enables them to move up the gradient.

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